Low birth weight increases susceptibility to renal injury in a rat model of mild ischemia-reperfusion

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Ojeda NB. Low birth weight increases susceptibility to renal injury in a rat model of mild ischemia-reperfusion. Am J Physiol Renal Physiol 301: F420–F426, 2011. First published May 25, 2011; doi:10.1152/ajprenal.00045.2011.—Renal injury due to ischemia-reperfusion (I/R) is the major cause of acute kidney injury. Whether enhanced susceptibility to renal injury due to I/R can be programmed during fetal life is unknown. Epidemiological studies indicate that low birth weight (LBW) individuals are more susceptible to renal injury than normal birth weight (NBW) individuals. Thus, the aim of this study was to test the hypothesis that LBW is associated with an increased susceptibility to renal injury induced by mild renal I/R (15-min ischemia). Systemic and renal hemodynamic parameters were determined in NBW and LBW adult male rats after mild renal I/R; renal superoxide production and tubular injury were also assessed. A subgroup was pretreated with tempol, a superoxide dismutase mimetic, initiated 15 min before ischemia. Mild renal I/R did not alter renal hemodynamic parameters, induce tubular injury, or induce superoxide production in NBW rats. However, renal hemodynamic parameters declined, superoxide production increased, and histological indicators of tubular injury were present following mild renal I/R in LBW rats. Acute treatment with tempol prevented these alterations in LBW rats subjected to mild renal I/R. Thus, these findings suggest that adverse conditions during fetal life can compromise the renal response to subtle insults leading to an increased susceptibility to renal injury, suggesting that LBW individuals may be an “at risk” population for renal disease. Additionally, the outcome of tempol treatment proposes a possible mechanistic pathway involved in mediating enhanced susceptibility to renal injury programmed during fetal life.

LOW BIRTH WEIGHT (LBW) defined as weight at birth equal or lower to the 10th percentile for a full-term newborn is a sensitive marker for an adverse fetal environment (21). The theory of developmental programming proposes that exposure to an insult during a critical period of early development can lead to survival adaptive changes in the fetus, but it can also result in long-term consequences, which increases the risk for development of disease later in life (8–10). A reduction in nephron number is observed in several animal models of LBW, suggesting that alterations in kidney structure may be programmed in response to fetal insult (11, 13, 31, 32, 47, 48, 58). Epidemiological and experimental studies indicate that progression of renal disease is more severe in LBW individuals relative to normal birth weight (NBW) individuals, suggesting that exposure to an adverse fetal environment may also program the kidney for an abnormal response to a secondary insult in later life (14, 19, 41).

Renal ischemia-reperfusion (I/R) injury is a critical life-threatening condition (1) and is a major cause of acute kidney injury (AKI). AKI carries a high mortality in humans (24, 26) and many forms of chronic kidney disease involve an ischemic component in the development of kidney injury (15, 36, 44). A significant reduction in renal blood flow (RBF) is the primary cause of renal ischemia and can occur during vascular surgery, organ procurement and transplantation, lowering of systemic blood pressure, and in renal vascular diseases (15). Ischemia typically damages the renal tubular epithelial cells and glomerular cells, and it is also characterized by several hallmark features at the cellular level that end in necrosis and/or apoptosis (56). The time of exposure to ischemia is a key factor in mediating the severity of the damage (4). Subtle insults such as 15 min of renal ischemia do not compromise renal structure and function in normal individuals as previously reported in Sprague-Dawley rats and humans (4, 53). However, whether an increased susceptibility to renal injury is observed in response to a subtle insult such as mild ischemia in LBW individuals is not yet known.

Intracellular and molecular mechanisms involved in the development of renal I/R injury are complex and not totally understood (15). Increased oxidative stress, exacerbated inflammatory response, and abnormal vascular response are commonly mentioned as contributors to cell injury and death in response to renal I/R (23, 30, 49). LBW condition is also associated with increased oxidative stress, inflammation, and vascular dysfunction (28, 29, 54). Therefore, the present study was designed to test the hypothesis that LBW is associated with an enhanced susceptibility to renal injury in response to mild renal I/R; and whether oxidative stress contributes to the enhanced susceptibility to a subtle renal insult.

METHODS

All experimental procedures proposed in this study were performed in accordance with National Institutes of Health guidelines for use and care of animals with approval of all protocols by the Animal Care and Use Committee at the University of Mississippi Medical Center, as described previously (2). Briefly, rats were housed in a temperature-controlled room (23°C) with a 12:12-h light-dark cycle with food and water available ad libitum. Timed pregnant Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN). At day 14 of gestation, rats destined for reduced uterine perfusion were clipped as described below. All dams were allowed to deliver at term with offspring’s birth weight recorded within 12 h of delivery. At this time, the number of pups in the control and reduced uterine perfusion litter was culled to eight pups per dam to ensure equal nutrient access for all offspring. Animals were weighed twice weekly. Pups were weaned at 3 wk of age. Adult male rats from 11 control pregnant dams and 10 reduced
uteren perfusion pregnant dams were randomly assigned into 8 groups: NBW-sham (n = 7), NBW I/R (n = 7), NBW-sham + tempol (n = 6), NBW I/R + tempol (n = 6) from control pregnant rats, and LBW-sham (n = 7), LBW I/R (n = 9), LBW-sham + tempol (n = 8), LBW I/R + tempol (n = 9) from reduced uterine perfusion pregnant rats.

Reduced uterine perfusion in the pregnant rat. Using the method previously described, reduced placental perfusion was used to induce LBW (2, 38). Briefly, all rats undergoing surgical procedures were anesthetized with 2% isoflurane (W.A. Butler, Memphis, TN) delivered by an anesthesia apparatus. At day 14 of gestation, a silver clip (0.203-mm ID) was placed around the abdominal aorta above the iliac bifurcation. To avoid compensation of blood flow from the ovarian arteries, silver clips (0.100-mm ID) were placed on both branches of the ovarian arteries. Pregnant rats not exposed to surgical procedure were used as the control pregnant counterpart group.

I/R renal injury. Adult male rats at age of 24 wk were exposed to bilateral mild I/R as previously described (4). Briefly, with animals under isoflurane anesthesia, bilateral mild renal I/R was induced by occluding both renal pedicles with microvascular clamps for 15 min (mild ischemia). Completeness of ischemia was verified by blanching of the kidneys which is indicative of stoppage of blood flow. The blood flow to the kidneys was reestablished by removal of both clamps (reperfusion) with visual verification of blood return by changing in the kidney’s color to a homogeneous dark pink. Animals subjected to the same procedure except the renal pedicles not clamped were used as sham. After mild renal I/R procedure, animals were instrumented for renal hemodynamic measurements as described below. The abdominal cavity was closed in two layers (muscles and skin) and the animals were placed in separate cages for recovery.

The body temperature of the animals was monitored and maintained stable during the whole procedure using a rectal thermometer in sync with a heating pad.

Drug administration. The SOD mimetic tempol was administered at a dose of 30 mg/kg by bolus infusion 15 min before ischemia in 3-ml volume.

Measurement of systemic and renal hemodynamics. As previously described (2), rats under isoflurane anesthesia were surgically instrumented with flexible catheters (PE 90 tubing) in the right jugular vein for infusion and in the right carotid artery for measurement of arterial pressure and collection of blood; the bladder was also instrumented with a flexible catheter (PE 90 tubing) for collection of urine. All catheters were tunneled to the nape of the neck and exteriorized. Renal function and arterial pressure measurements were performed in the conscious state after a 2-h recovery phase from the ischemic event. Mean arterial pressure (MAP) was monitored in conscious, chronically instrumented rats via connection of the arterial catheter to a pressure transducer and a data set (PowerLab 16/30) using the software Lab Chart Pro V7 both from ADInstruments. The data-acquisition set was connected to a PC for continuous recording. Glomerular filtration rates (GFR) and effective renal plasma flow (eRPF) were calculated from radioactivity of $^{125}$I-iodotamale and concentration of para-aminohippuric acid (PAH), respectively, in plasma and urine. Renal vascular resistance (RVR) and filtration fraction (FF) were calculated using the following: RVR = (MAP/eRPF) × (1 − hematocrit) and FF = (GFR/eRPF), respectively. Data were collected during 20-min clearance after rats reached a steady-state condition. IT25 activity was calculated using a gamma counter and PAH concentration was determined colorimetrically.

Measurements of renal superoxide production. Lucigenin chemiluminescence was utilized for detection of basal superoxide and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase derive superoxide production in kidney tissues. Spontaneous superoxide generation and NADPH-oxidase derivate superoxide production were measured in kidney cortical homogenates using the lucigenin chemiluminescence method in a luminometer (Autolumat Plus 953), as previously described by Schluter et al. (22, 45). Protein concentrations in the kidney homogenates were determined by the method of Lowry et al. The data are expressed as relative light units per milligram protein.

Tissue morphology. Right kidneys were collected for histological assessments from each group of rats. After collection, kidneys were placed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned (4-μm thickness) and then stained with hematoxylin and eosin (Suripath Medical Industries, Leica, IL) (40). The evaluations of the slides were performed in a blinder manner to samples identity, using a modified semiquantitive evaluation. The method used for tissue scoring is based on random observation of 10 fields per each examined kidney (high-power fields). The magnitude of tubular cell epithelial edema, cell loss, necrosis, intratubular debris, and tubular cast formation was scored into five levels on the basis of the percentage of affected tubules in a high-power field under light microscope: 0, none; 1 <25%; 2, 25 to 50%; 3, 50 to 75%; 4 >75%, as described elsewhere (27).

Statistics. GraphPad PRISM version 5 was used for all statistical analysis. When comparison was made between groups, ANOVA with adjustments for multiple comparisons was used. A value of P < 0.05 was considered statistically significant.

RESULTS

Birth weight, body weight, and kidney weight in LBW rats. Offspring from pregnant rats exposed to reduced uterine perfusion had a lower birth weight compared with offspring delivered from control pregnant rats. However, by 24 wk of age no significant difference in body weight was observed upon comparison of LBW to NBW; kidney weight and kidney weight adjusted to body weight also did not differ at 24 wk of age (Table 1).

### Table 1. Body and kidney weight

<table>
<thead>
<tr>
<th>Groups</th>
<th>Birth Body Weight, g</th>
<th>24 Wk of Age Body Weight, kg</th>
<th>Kidney Weight, g</th>
<th>Kidney/Body Weight Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBW sham</td>
<td>6.59 ± 0.04</td>
<td>0.487 ± 0.19</td>
<td>3.58 ± 0.25</td>
<td>0.73 ± 0.09</td>
</tr>
<tr>
<td>LBW sham</td>
<td>5.37 ± 0.07*</td>
<td>493 ± 25</td>
<td>3.15 ± 0.16</td>
<td>0.64 ± 0.08</td>
</tr>
<tr>
<td>NBW I/R</td>
<td>6.55 ± 0.10</td>
<td>427 ± 41</td>
<td>3.73 ± 0.59</td>
<td>0.86 ± 0.14</td>
</tr>
<tr>
<td>LBW I/R</td>
<td>5.31 ± 0.06*</td>
<td>410 ± 28</td>
<td>3.93 ± 0.17</td>
<td>0.98 ± 0.23</td>
</tr>
<tr>
<td>NBW sham tempol</td>
<td>6.84 ± 0.09</td>
<td>458 ± 37</td>
<td>2.98 ± 0.10</td>
<td>0.66 ± 0.10</td>
</tr>
<tr>
<td>LBW sham tempol</td>
<td>5.67 ± 0.12*</td>
<td>467 ± 31</td>
<td>2.92 ± 0.23</td>
<td>0.66 ± 0.12</td>
</tr>
<tr>
<td>NBW I/R tempol</td>
<td>6.67 ± 0.11</td>
<td>469 ± 36</td>
<td>2.95 ± 0.39</td>
<td>0.61 ± 0.14</td>
</tr>
<tr>
<td>LBW I/R tempol</td>
<td>5.55 ± 0.15*</td>
<td>427 ± 22</td>
<td>3.00 ± 0.13</td>
<td>0.71 ± 0.13</td>
</tr>
</tbody>
</table>

All data are expressed as means ± SE. Body and kidney weight. Body weight was measured at birth and at 24 wk of age. Kidney weight was measured at 24 wk of age. Kidney/body weight ratio was calculated using body weight and kidney weight at 24 wk of age. *P < 0.05 vs. normal birth weight (NBW). LBW, low birth weight; I/R, ischemia-reperfusion.
Effect of mild I/R on systemic and renal hemodynamic. MAP was significantly elevated in LBW rats compared with NBW rats under sham conditions ($P < 0.05$) and after exposure to mild renal I/R ($P < 0.05$). Mild renal I/R had no effect on MAP in NBW or LBW rats. (Fig. 1). Renal hemodynamic parameters including RVR (Fig. 2), eRPF (Fig. 3), RBF (Fig. 4), and GFR (Fig. 5) did not differ on comparison between LBW and NBW sham rats. However, in response to mild renal I/R, or 15 min of ischemia followed by 2-h reperfusion, RVR was significantly increased in LBW ($P < 0.05$) compared with NBW rats; eRPF, RBF, and GFR were significantly decreased in LBW rats ($P < 0.05$) compared with NBW rats. FF did not show differ following mild I/R in LBW or NBW rats (Fig. 6). Moreover, mild renal I/R did not induce any change in renal hemodynamic parameters in NBW rats.

Effect of mild I/R on renal superoxide production. Basal superoxide production and NADPH-oxidase derivate superoxide production were significantly elevated in LBW rats compared with NBW rats under sham conditions ($P < 0.05$) and after exposure to mild renal I/R ($P < 0.05$). After exposure to mild renal I/R, LBW rats showed significant increments in renal superoxide production compared with NBW rats under mild renal I/R conditions and to LBW rats under sham conditions ($P < 0.05$). Moreover, mild renal I/R did not affect renal superoxide production in NBW rats.

Effect of the SOD mimetic tempol on systemic and renal hemodynamic parameters. Pretreatment with tempol 15 min before mild renal I/R prevented the altered renal hemodynamic responses observed in LBW rats. Specifically, pretreatment with tempol attenuated the marked increases in RVR (Fig. 2) and the significant reduction in eRPF (Fig. 3), RBF (Fig. 4), and GFR (Fig. 5) observed in LBW exposed to mild renal I/R. Treatment with tempol had no effect on MAP in LBW offspring; moreover, treatment with tempol had no effect on systemic and renal hemodynamic parameters in NBW rats. In addition, FF was not altered by pretreatment with tempol in either LBW or NBW rats (Fig. 6).

Effect of SOD mimetic tempol on renal spontaneous superoxide and NADPH-oxidase derivate superoxide production. Pretreatment with tempol 15 min before mild renal I/R prevented further increments of renal basal superoxide production and NADPH-oxidase derivate superoxide production; al-
though, it failed to normalize them to values comparable to NBW rats (Fig. 7, A and B).

Tissue morphology. The kidney sections from NBW rats exposed to sham and mild renal I/R and LBW rats exposed to sham procedure showed structural indemnity in tubules segments. However, the kidney sections from LBW rats exposed to mild renal I/R show cellular edema, epithelial cell loss, intratubular debris, and tubular cast formation. These findings show significantly more tubular injury in LBW rats exposed to mild renal I/R (injury score 3.4 ± 0.2, *P < 0.05 vs. all other groups). Tempol-treated groups did not show any histological evidence of tubular damage either in NBW or LBW rats exposed to sham or mild renal I/R (Fig. 8, A and B). The method used for tissue scoring is based on random observation of 10 fields per each examined kidney (high-power fields). The pictures were taken at ×400 magnification (scale bars = 50 μm).

DISCUSSION

The main findings from this study are that LBW rats exhibit 1) altered renal hemodynamic parameters, 2) increased superoxide production, and 3) changes in histological morphology, suggesting acute tubular damage after exposure to a mild (15 min) renal ischemia followed by 2 h of reperfusion. Importantly, 4) LBW rats did not show alterations in renal function, oxidative stress, or changes in tubular structure when exposed to the same mild renal I/R. These findings are indicative of an increased susceptibility to renal injury associated with LBW. Additionally, 5) pretreatment with the SOD mimetic, tempol, prevented the changes in renal hemodynamic parameters, indications of tubular injury, and increases in oxidative stress markers in LBW rats, suggesting a potential mechanistic pathway with oxidative stress playing a key role in the increased susceptibility to mild renal I/R observed in LBW rats.

The rat model of LBW used in this study develops hypertension as early as 4–6 wk of age (2); however, impairment in renal function is not observed under basal conditions as was found in our current study and accordingly with previous reports by Alexander (2). This finding suggests that renal function is preserved in LBW rats by overloading the kidney’s reserve to maintain the body’s physiological requirements. LBW rats’ kidneys may become more susceptible to damage with a slight secondary hit that would not have consequences in NBW rats. Our findings demonstrate that a subtle insult such as 15 min of bilateral renal ischemia results in renal functional and structural changes indicative of renal injury in LBW rats. Renal hemodynamic parameters were measured 2 h after release of the vascular clamps used to induce bilateral renal ischemia. Renal parameters were altered only in LBW rats exposed to mild bilateral renal ischemia marked by elevations.
Oxidative stress has been reported in several studies as an important factor involved in I/R injury (23, 30, 49). I/R markedly increases the production of ROS including superoxide anion ($O_2^-$), hydroxyl radicals, hypochlorous acid, hydrogen peroxide ($H_2O_2$), and peroxynitrite (23). ROS may play a role in renal cell injury due to ischemia shifts in the cellular metabolism from aerobic to anaerobic resulting in an intensification of $O_2^-$ and $H_2O_2$ generation (30). Mitochondria is one of the major sources of ROS associated with I/R to induce lipid peroxidation of cell membranes, protein, and enzyme oxidation, and DNA damage leading to apoptotic and necrotic cell death (18, 37, 51). Traditionally, ROS generated during I/R are considered to produce cell damage via a direct action on target cells (23, 30); however, it is now apparent that ROS can also act as signal transduction molecules to regulate gene transcription, activate multiple transcription factors, and also trigger an inflammatory response (5, 17) with deleterious consequences (15). ROS also can contribute to RBF autoregulation as reported by other investigators (25, 50) affecting another factor related to I/R renal injury, renal autoregulation. LBW is associated with an increment in the production of ROS, an observation reported in animal models of fetal programming and in LBW individuals (33, 52). Reperfusion occurs upon restoration of RBF and is essential for preventing ischemic cell death. However, reperfusion can contribute by itself to cell injury and death due to the reperfusion injury phenomenon. During reperfusion, $O_2$ is added abruptly, which leads to increment free $O_2^-$ and more $H_2O_2$ and ROS production increasing oxidative stress during the reperfusion phase (15, 56). Whether oxidative stress plays a role in mediating AKI in LBW exposed to mild renal I/R has not been elucidated to date. The participation of the NADPH-oxidase system in generating increased levels of superoxide in LBW rats is strongly suggested based on our findings. Therefore, further examination of NADPH-oxidase activity in addition to uncoupling of eNOS as potential mediators of increased oxidative stress in LBW rats is currently under investigation.

The role of renal sympathetic nervous system (SNS) and circulating catecholamines in the pathogenesis of I/R-induced renal injury has been described before (7, 20). During ischemia, the afferent renal nerve is activated leading to reflex activation of the efferent sympathetic renal nerve by a renal chemoreceptor (35, 42, 43). Also, norepinephrine overflow into renal vein was markedly increased during reperfusion phase and lasted for 24 h after reperfusion (20). The ATP depletion induced by ischemia could lead to accumulation of axoplasmic norepinephrine that is massively released during the reperfusion phase (46). Additionally, renal denervation or ganglion blocker ameliorated renal dysfunction and tissue damage in kidneys exposed to I/R (20). The importance of the renal SNS cannot be ruled out in this model of LBW that exhibits marked susceptibility to mild renal I/R insult. Previously, we reported renal SNS involvement in intrauterine growth restriction-induced hypertension in LBW rats (3, 39). The current study shows that pretreatment with tempol prevents the decline in renal function induced by mild renal I/R in LBW rats. Tempol systemically administered may mediate its effects via central nervous neurons to reduce renal sympathetic nerve activity as previously reported (55). Therefore, the involvement of renal SNS in the increased susceptibility to renal injury observed in LBW can be suggested. Our findings are similar to others in LBW and reductions in eRPF, RBF, and GFR. Acute tubular damage has been previously observed by others in a model of early monitoring of tubular necrosis in the rat, utilizing Na23 magnetic resonance imaging after just 1 h of renal reperfusion period (6). In this study utilizing the mild I/R protocol, renal structural changes were observed in kidneys of LBW rats exposed to the mild I/R protocol and harvested at the end of the 2-h reperfusion period. Thus, the novelty of these findings points to the unexplored field of susceptibility to renal injury induced by subtle early life insults associated with LBW. Additionally, the responses to tempol treatment observed in LBW rats exposed to mild I/R open the field to investigate possible mechanism(s) involved in the enhanced susceptibility of exposed LBW rats to renal injury. Whether ischemic renal injury induced in LBW rats follows the same mechanistic pathways already explored in NBW rats is unknown and merits further investigations.

The intracellular and molecular mechanisms involved in the development of renal I/R injury are complex and not totally understood (15). Three factors have been identified related to renal injury due to I/R: an increased production of reactive oxygen species (ROS), an inflammatory response, and renal vascular abnormalities associated with vasoconstriction in the reperfusion phase, all contributing to renal cell injury and death (18, 23, 30, 37, 49, 51).
which tempol treatment prevents renal damage in a model of renal I/R injury by reducing sympathetic activity (16, 57). However, SNS involvement in LBW susceptibility to renal injury should be examined in depth with further studies to explore this interesting pathway.

Hypertension observed in this model of LBW could be another factor involved in the renal susceptibility to I/R injury. The major cause of renal injury induced by hypertension is related to the transmission of high pressure to the glomerular and peritubular beds in the outer medulla where RBF autoregulation is relatively ineffective (34). The vasoconstriction of the afferent arterioles in response to increases in systemic blood pressure appears to be impaired in individuals with hypertension and other chronic kidney diseases, suggesting a long-term effect (12).

A pressure-related factor involved in the susceptibility to I/R renal injury is debatable in our model based on the fact that tempol treatment prevented alterations in renal functions in LBW rats, yet it had no effects on lowering blood pressure to levels comparable with NBW rats. Additionally, MAP between LBW and NBW rats exposed to I/R did not show significant differences. However, additional investigations will be conducted to investigate the involvement of hypertension in the susceptibility to renal injury in this particular model. A protocol using chronic antihypertensive therapy that does not include drugs affecting the RAS or SNS will be suitable to explore hypertension involvement in this model.

In conclusion, findings from this study suggest that LBW is a risk factor associated with an increased susceptibility to renal injury in response to a subtle insult such as mild ischemia. Therefore, the risk factors to develop renal diseases may be underestimated in LBW individuals, which may delay early intervention to prevent kidney injury and the progression of kidney disease.

Perspectives

This study proposes that subtle insults that have no effects on NBW individuals may lead to renal injury in LBW individuals. These results strongly suggest a critical role for influences during fetal life on later risk for renal diseases. It is well-documented that adverse events during fetal life have long-term consequences showing poor outcomes with any interventional strategy. However, better understanding the early origins of adult diseases will help to identify LBW individuals as “at risk population” and may lead to the development of preventive strategies to enhance their long-term health and reduce the risk to develop diseases later in life.

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